IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of:

Marie-Cecile van de Lavoir

Serial No.: 10/067,148

Filed: February 1, 2002

For: CHIMERIC BIRD FROM EMBRYONIC STEM CELLS

Group Art Unit: 1636

Examiner: Sumesh Kaushal

#10

THIRD INFORMATION DISCLOSURE STATEMENT

Commissioner for Patents
Washington, D.C. 20231

Sir:

In accordance with 37 CFR §§ 1.97 and 1.98, the items identified in this Information Disclosure Statement ("IDS") are brought to the attention of the Office. The items are listed on the attached form PTO-1449 and copies are enclosed for the convenience of the Examiner.

The items identified in this IDS may or may not be "material" pursuant to 37 CFR § 1.56. The submission thereof by Applicant is not to be construed as an admission that any such patent, publication or other information referred to therein is material or considered to be material (37 CFR § 1.97(h)), or even qualifies as "prior art" under 35 USC § 102, § 103, or § 135 with respect to this invention unless specifically designated by Applicant as such.

Regarding the documents cited in the PTO Form 1449 submitted herewith, the following comments address the references in the order listed on the 1449.

CERTIFICATE OF HAND DELIVERY

I hereby certify that this paper (along with any referred to as being attached or enclosed) is being hand delivered to EXAMINER SUMESH KAUSHAL AT CM-1, 7TH FLOOR, FRONT DESK, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Date of Deposit: 6/20/2003

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U.S. Patent Documents:

6,333,192, Petitte et al., issued on December 25, 2001, "Method of Producing an Undifferentiated Avian Cell Culture Using Avian Primordial Germ Cells."

This reference discloses cells expressing an embryonic stem cell phenotype derived from primordial germ cells. Chimeras from embryonic stem cells are not demonstrated experimentally.

6,114,168, Samarut et al., issued on September 5, 2000, "Active Retinoic Acid-Free Culture Medium for Chicken Embryonic Stem Cells."

The disclosure of two references relating to avian embryonic stem cells includes the following:

> A very great advance in the production of transgenic animals has been brought about in mice by the development of ES cell technology.
>
> ES cells (embryonic stem cells) are totipotent embryonic cells capable of regenerating all the tissues of the embryo, including the germ tissue, after their injection into very early embryos. These cells may hence be considered to be Trojan horses for introducing new genetic information into an animal's genetic constitution. The possibility of culturing these cells in the long term in vitro affords the possibility of exercising numerous controls before their implantation in vivo. Moreover, these cells may be stored without limit in liquid nitrogen, which constitutes a potential for storage of a genetic constitution.

Col. 1, line 59 – Col. 2, line 5.

* * * *

> The use of ES cells nowadays constitutes the most promising approach in domestic birds for the efficient production of transgenic animals.
>
> Recent work from a Canadian group (R. Etches at the Guelph station) has suggested that ES cells must exist in the bird embryo (Petitte et al., 1990). This group at [sic] succeeded in transplanting such cells into embryos and consequently producing animals whose genetic constitution is derived from that of the grafted cells. However, to date, it has not been possible for success to be achieved in culturing these cells in vitro; as a result, it has not been possible to use these cells to transfer a transgene in a stable manner. This is a major impediment to the exploitation of ES cell technology in birds. ES cells may be characterized by three essential types of criteria:
>
> morphology

endogenous alkaline phosphatase activity

reaction with antibodies which are specific for a state of totipotency (ECMA-7, SSEA-1 and SSEA-3, in particular).

To date, it has not been possible to obtain any culture of ES cells which are identified by these collective characteristics. Col. 2, lines 26-27.

5,830,510, Petitte et al., issued on November 3, 1998, "Veterinary Pharmaceutical Formulation Containing Avian Embryonic Stem Cells;"

5,656,479, Petitte et al., issued on August 12, 1997 "Avian Embryonic Stem Cells;" and

5,340,740, Petitte et al., issued pm August 23, 1994, "Method of Producing an Avian Embryonic Stem Cell Culture and the Avian Embryonic Stem Cell Culture Produced by the Process."

Each of these references discloses an embryonic stem cell culture. Chimeras from embryonic stem cells are not demonstrated experimentally.

Foreign Patent Documents:

WO 96/12793, Samarut et al., filed October 20, 1995, "Active Retinoic Acid Free Culture Medium for Avian Tutipotential Embryonic Stem Cells."

This substantive content of this reference is similar in content to Pain et al. "Long-term In vitro Culture and Characterisation of Avian Embryonic Stem Cells with Multiple Mophogenetic Potentialities," Development 122:2339-2348, (1996) cited below. Although the WO reference does not disclose the duration of the embryonic stem cell culture as noted below, the journal article describes chimeras created from a 19-day old ES cell culture.

This publication is substantially identical in content with the USP documents 5,830,510, 5,656,479, and 5,340,740 above.

WO 90/01541, Williams et al., filed August 3, 1989, "*In Vitro* Propagation of Embryonic Stem Cells."

This publication is the earliest known reference to disclose culture techniques for chicken embryonic stem cells and discloses sustained ES cell cultures *in vitro*. Chimeras from embryonic stem cells are not demonstrated experimentally.

Other Documents:

Ivarie, Robert, "Avian transgenesis: progress towards the promise," Trends in Biotechnol. Vol 21, No. 1 p. 14-19, Jan. 2003.

This reference is not prior art to the pending claims, but recites the author's view of the state of the art relative to the present invention. The author states:

> **The search for chick embryonic stem (ES) cells**
>
> An intensive effort for more than a decade has been made to isolate and exploit avian ES cells from the stage X blastoderm containing pluripotent cells. For example, blastodermal cells from black feathered Barred Rock embryos injected into the subgerminal cavity of recipient White Leghorn embryos generated a high frequency of black and white feather chimeras, many of which transmitted the black allele to G1 progency [23-25, 65]. When cells were transfected with a reporter gene before injection, cells expressing the reporter could be identified in practically all structures of the developing embryo [24, 65]. When cultured, the cells eventually took on a phenotype and differentiated into a variety of cell types typical of mouse ES cells [66]. To date, however, no transgenic G0 birds have been generated using this procedure because the cells lose their germline competence when cultured. p.17, col. 1, 2nd ¶.

Petitte, James N. "The Avian Germline and Strategies for the Production of Transgenic Chickens," J. Poultry Science, 39: 205-228, July 2002

This reference is not prior art to the pending claims, but recites the author's view of the state of the prior art relative to the present invention. The author states:

> Currently, transgenic poultry can be produced using retroviral vectors and through the microinjection of DNA into the germinal disk of the newly fertilized egg. However, other approaches such as the development of embryonic stem cells, the culture of primordial germ cells, and sperm-mediated transfection are being explored for their potential to produce transgenic poultry. p. 205, 1st ¶.
>
> * * * * *
>
> During the last ten years, progress in the development of avian embryonic stem cells appears to have been slow because the culture conditions required for the production of avian embryonic stems cells is unknown. Nevertheless, various attempts have been made to culture blastodermal cells of PGCs so that they could be used for the production of transgenic poultry. Etches et al. (1996) compared the culture of blastodermal cells are intact blastoderms, dispersed blastodermal cells culture in a monolayer or with a confluent layer of mouse fibroblasts. p. 220, 3rd ¶.
>
> * * * * *
>
> Currently, no reports exist on the development of transgenic birds using avian embryonic stem cells or culture PGCs. The main attraction of continuing to work on this approach is that culture stem cells and germ cells remain the only means available to develop transgenic chickens with specific changes to the genome using homologous recombination. Properly designed "gene targeting" vectors could make the function of the resulting transgenic more predictable and avoids the need to screen several individuals for proper gene expression. Few of the transgenic chickens developed with retroviral vectors or microinjection of DNA resulted in good gene expression. This indicates that the effort to utilize embryonic stem cells is worthwhile. p. 221, 2nd ¶.

Zajchowski, L.D. and Etches, Robert, "Transgenic Chickens: Past, Present and Future," Poultry and Avian Biology Reviews 11(2):63-80 2000.

This reference describes ES-cell derived chimeras from 7-day old cell cultures:

> Transgenesis via the use of the chimeric intermediates constructed with primordial germ cells or blastodermal cells has been proposed to allow precisely designed gene targeting experiments to be carried out. While it is now possible to routinely produce chimeras using both primordial germ cells and blastodermal cells, long-term culture techniques which would permit the genetic modification of

blastodermal or primordial germ cells by homologous recombination *in vitro* are not yet available. Overall, despite significant progress, much research is still required in order to establish practical, efficient and economical techniques for the production of transgenic chickens. p. 63, Abstract.

While somatic and germline chicken chimeras are now routinely produced by the transfer of freshly isolated blastodermal cells, the ability to maintain pluripotential blastodermal cell populations in culture remains limited. Chicken blastodermal cells have been cultured for up to 48 hours as explanted intact embryos, as dispersed cells in a monolayer, and as dispersed cells on a confluent layer of mouse fibroblasts (Etches *et al* 1996). For all three culture conditions, both somatic and germline chimeras were produced following injection into irradiated recipient Stage X blastoderms. Culture of blastodermal cells on a feeder layer of mouse fibroblasts, commonly used in murine embryonic stem cell culture, led to a higher frequency of somatic chimeras with a greater extent of feather plumage derived from donor cells when compared to intact explants or monolayer cultures. The ability of cultured cells to contribute to the germline was no different under the three culture systems used. However, both the frequency and extent of somatic and germline chimerism were significantly reduced when cells cultured for 48 hours were used in place of freshly isolated cells. Pain *et al.* (1996) characterized putative avian embryonic stem cells which were maintained in culture on a mouse feeder layer in a medium containing a complex cocktail of growth factors for at least 35 passages (more than 160 days). These cells were morphologically similar to murine ES cells, expressed immunohistochemical markers characteristic of ES cells, and could be induced to differentiate *in* vitro into various cell types including muscle, hematopoietic and nerve cells. Long-term proliferation was dependent on the presence of LIF in the medium. To test the ability of the cultured cells to contribute to somatic and germline lineages in chimeras, cells cultured for 1-3 passages (cells were passaged after 4-7 days) were injected into recipient blastoderms. Regardless of passage number, more than 50% of the hatched chicks exhibited somatic chimerism. However, cells could only be cultured for up to 7 days before the ability to contribute to the germline was lost. Of a large number of chimeras tested, two chimeric chickens derived from 7-day old cultures demonstrated germline transmission of donor-derived cells. Although there are other reports of long-term cultures of blastodermal cells under various conditions, they provide no evidence of the ability of the cultured cells to contribute to

> chimeras *in vivo* (Petitte and Yang, 1993; Liu, 1995; Tsai *et al*, 1995). p. 74, col. 1-2.
>
> * * * * *
>
> However, realization of the full potential of the chimera approach awaits the establishment of long-term culture methods for PGCs or blastodermal cells. Overall, much research is still required for the development of practical and economical techniques for accessing the avian genome. If this technology becomes available in the future, transgenic chickens could be of enormous benefit in the laboratory and on the farm. p. 76, Col. 1 Conclusion.

Pain et al., "Chicken Embryonic Stem Cells and Transgenic Strategies," Cells Tissues Organs, 165:212-219 (1999).

This reference describes "ES-like cells" as follows:

> We have successfully isolated ES-like cells from chicken, and suggest that similar techniques can be applied to isolate ES-like cells from avian species. Identifying the best conditions to efficiently transfect these cells will be the key to the ultimate success in generating transgenic birds. Further work is however needed to determine their contribution to somatic cells and to evaluate the germ-line competence of these cells which is currently very low. p. 218, col. 2, Conclusion.

Pain et al., "Long-term in vitro culture and characterisation of avian embryonic stem cells with multiple mophogenetic potentialities," Development 122:2339-2348, (1996).

The authors describe short-term cultures of chicken ES cells and the creation of chimeras from cells culture up to 19 days. The authors state:

> The ability of long-term cultures to give rise to chimeric animals is currently under investigation. p. 2344, col 2., 2nd ¶.
>
> * * * * *
>
> Work is in progress to improve the culture conditions allowing clonal selection of stem cells and further work is required to improve donor-derived cells so that they can enter readily and at a high frequency into the germ line. p. 2346, col. 2. end of 1st ¶.

Referring to the legend for Figure 8, 19 days is the longest reported duration of an ES cell culture from which chimeras were produced.

Sang, "Transgenic chickens – methods and potential applications," Trends in Biotechnol. 12:415-420 (1994).

The author notes the promise of transgenic chickens derived from ES cells and mentions some of the technical requirements.

> One of the most exciting prospects for the genetic manipulation of chickens is the potential development of an embryonic stem (ES)-cell system. This would enable not only the addition of gene constructs, but also gene targeting to alter endogenous genes. Such a system requires three components: (1) the culture of pluripotential cell lines; (2) transfection and selection of transfected cells; and (3) introduction of selected embryonic cells into recipient embryos and their incorporation into the germline. The ability to make chimaeric embryos by the injection of dispersed blastodermal cells into recipient embryos, resulting in birds with chimaeric gonads, is the first of these requirements to have been achieved, by Gibbins, Etches and colleagues.[16] Irradiation of recipient blastodermal embryos *in ovo*, prior to injection of donor cells, resulted in the production of somatic chimaeras at a high frequency (58%), with a high proportion of germ-line chimaeras. The isolated blastodermal cells can be transfected efficiently by lipofection, but transfected cells have yet to be shown to be transmitted through the germ line[17]. Several laboratories are developing culture systems for chick-embryo cells, but it is not possible to predict if stable ES cells that can be grown for long periods in culture to allow for selection for targeted recombination events can be maintained easily. p. 417, 1st ¶.

Perry et al., "Transgenesis in Chickens,"Transgenic Research 2:125-133 (1993).

The authors describe alternate methods for manipulating the avian genome and conclude:

> In the long term, the establishment of pluripotent cell lines to enable the selection of cells of the desired genotype for transfer to host embryos would appear to offer the ideal solution to the problem. p. 131, col. 1, end of ¶.

Etches et al., "Novel approaches to studies of avian endocrinology using transgenic chickens produced via chimeric intermediates," In Avian Endocrinology, Edited by P.J. Sharp, pp. 381-396, (1993) J. Endocrinology, Bristol, UK.

This reference describes strategies for producing transgenic chickens, including the use of compromised recipients.

Etches, R.J., "A Genetic Approach to Physiology," Chapter 1, pp. 1-13 in "Manipulation of the Avian Genome" edited by R.J. Etches and A.M. Verrinder Gibbins (1993), CRC Press, Boca Raton

The author states:

> In chickens a pluripotential cell that is capable of replication without differentiation *in vitro* while retaining the ability to enter the germline following injection into a recipient embryo has not been identified. ... It is clear therefore that although the use of chimeras to mediate genome manipulation has many theoretical advantages, its realization will depend on the development of embryonic stem cell lines. p. 7, 3rd ¶.

Shuman, "Production of Transgenic Birds," Experientra, 47 897-905 (1991).

The author states:

> There are several groups currently attempting to produce embryonic stem cells for the chicken. Embryonic stem cells would have the advantage that donor cells could be selected prior to transfer and they could also be used for homologous recombination to allow targeted insertion. Cells lines of this type would have tremendous value for gene transfer studies. To date, however, there are no reports of bird embryonic stem cells. The main limitation of using embryonic stem cells, or chimeras in general, is the low number of progeny produced from the transferred cells. In the future, it may be possible to alleviate this problem by devising strategies to reduce host germ cell populations. p. 903, col. 1, 3rd ¶.

Petitte et al., "Production of Somatic and Germline Chimeras in the chicken by Transfer of Early Blastodermal Cells,"Development 108: 185-89 (1990).

The authors state:

> Assuming that mouse and chicken germline chimeras are analogous, it should be possible to create transgenic chickens using a similar strategy to that described by Bradley *et al.* (1984) for the mouse. Development of these techniques would facilitate the use of homologous recombination and site-directed mutagenesis in studies where manipulation of the chicken genome was either the goal in itself or a means of introducing specific changes in gene expression in order to study the biology of embryonic development. p. 188, col. 1, 3rd ¶.

Gibbins, A.M. Verrinder et al., "Efficient Transfer of Chicken Blastodermal Cells and Their Incorporation Into Recipient Embryos to Produce Chimeric Chicks," Proceedings of the 4th World Congress on Genetics Applied to Livestock Production," Edinburgh July 23-27 (1990). Proceedings 16:119-122 (1990).

The authors state:

> A major problem that we have encountered is that we have not been successful in culturing chicken embryonic stem cells for any extended period of time without differentiation taking place. The establishment of embryonic stem cell lines has also proved difficult for researchers who are working with mammalian species other than the mouse. An added complication is that little success has ever been achieved in establishing permanent cell lines from birds, no matter what the issue of origin. However, we are still pursuing this goal vigorously. p. 903, col. 1, 3rd ¶.

Etches et al., " Production of Chimeric Chicks by Blastodermal Stem Cell Transfer and the Prospects to Gene Manipulation," Chapter 22:305-309, (1990). In *Avian incubation and embryology.* Edited by S. Tullett, 22nd British Poultry Science Symposium

The authors state:

> In the chicken, we are several years away from a similar ... (i.e. murine ES cell) ... application since we haven not yet established reliable methods for the culture of blastodermal stem cells nor have we demonstrated that cultured cells can contribute to the germline. p. 308, 2nd ¶.

Etches et al., " Poultry Genetics by the Year 2000," Chapter 22:305-309, (1990), pp 16-19

The authors illustrate the procedure of transfecting foreign DNA into embryonic stem cells maintained in culture, the formation of chimeras with embryonic stem cells and the identification of transgenic chickens among the offspring of chimeras. After describing the approach the authors state:

> Although we do not yet know how these transfected cells will act in recipient embryos, we are hopeful that they will integrate into the recipient in the same manner as did the non-transfected (and freshly isolated blastodermal cells). p. 16, col. 2, 1st ¶.

INFORMATION DISCLOSURE STATEMENT FILING PROVISION:

This IDS is submitted under 37 CFR § 1.97(b)(3), thus, no fee is required.

The Commissioner is authorized to charge any fees required by the filing of these papers, and to credit any overpayment to Orrick, Herrington & Sutcliffe's Deposit Account No. **150665**.

Respectfully submitted,

ORRICK, HERRINGTON & SUTCLIFFE LLP

Dated: June 19, 2003

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FORM PTO-1449 LIST OF PATENTS AND OTHER ITEMS FOR APPLICANT'S INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary)	ATTY. DOCKET NO. 700603.3	SERIAL NO. 10/067,148
	APPLICANT: Marie-Cecile van de Lavoir et al.	
	FILING DATE: February 1, 2002	GROUP: 1636

	✓	Etches, R.J., "A Genetic Approach to Physiology," Chapter 1, pp. 1-13 in "Manipulation of the Avian Genome" edited by R.J. Etches and A.M. Verrinder Gibbins (1993), CRC Press, Boca Raton
	✓	"Production of somatic and germline chimeras in the chicken by transfer of early blastodermal cells," Petitte, J.N. et al., Development 108, 185-189 (1990)
	✓	"Production of transgenic birds," Shuman, R.M., Experientia 47, 897-905 (1991)
	✓	"Efficient Transfer of Chicken Blastodermal Cells and Their Incorporation Into Recipient Embryos to Produce Chimeric Chicks," Gibbins, A.M Verrinder et al., Proceedings of the 4th World Congress on Genetics Applied to Livestock Production XVI, 119-122, Edinburgh: July 23-27, 1990
	✓	"Production of chimeric chicks by blastodermal stem cell transfer and the prospects for gene manipulation," Etches, R.J. et al., In Avian Incubation and Embryology, Edited by S. Tullett p. 305-309 1990, 22nd British Poultry Science Symposium, British Poultry Science, Edinburgh.
	✓	"Poultry Genetics by the Year 2000," Etches, R.J. et al., Department of Animal and Poultry Science, University of Guelph, published 1990

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U.S. PATENT DOCUMENTS

EXAMINER INITIAL		DOCUMENT NUMBER	DATE	NAME	CLASS	SUB CLASS	FILING DATE
		6,333,192	12/25/2001	Petitte et al.			
		6,114,168	9/5/2000	Samarut et al.			
		5,830,510	11/3/1998	Petitte et al.			
		5,656,479	8/12/1997	Petitte et al.			
		5,340,740	8/23/1994	Petitte et al.			

FOREIGN PATENT DOCUMENTS

EXAMINER INITIAL		DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUB CLASS	TRANSLATION YES	TRANSLATION NO
		WO 96/12793 ✓		PCT				
		WO 93/23528 ✓	1993	PCT				
		WO 90/01541 ✓	1989	PCT				

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)

	✓	"Avian transgenesis: progress towards the promise," Ivarie, Robert, Trends in Biotechnol. Vol 21, No. 1 Jan. 2003
	✓	"The Avian Germline and Strategies for the Production of Transgenic Chickens," Petitte, James N., J. of Poultry Science, 39: 205-228, July 2002
	✓	"Transgenic Chickens: Past, Present and Future," Zajchowski, L.D. et al., Poultry and Avian Biology Reviews 11(2):63-80 2000
	✓	"Chicken Embryonic Stem Cells and Transgenic Strategies," B. Pain et al., Cells Tissues Organs 1999; 165:212-219
	✓	"Long-term in vitro culture and characterisation of avian embryonic stem cells with multiple mophogenetic potentialities," Pain, B. et al., Development 122 2339-2348, 1996)
	✓	"Transgenic chickens – methods and potential applications," Sang, Helen, Trends in Biotechnol. 12:415-420 (1994)
	✓	"Transgenesis in Chickens," Perry, Margaret M. et al., Transgenic Research 2, 125-133 (1993)
	✓	"Novel approaches to studies of avian endocrinology using transgenic chickens produced via chimeric intermediates," Etches, R.J. et al., In Avian Endocrinology, Edited by P.J. Sharp, pp 381-396, 1993 J. of Endocrinology, Bristol, UK

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